Biogas production from Artichoke processing wastes by using semi- continuous feeding system.

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Artichoke processing wastes were used to evaluate the biogas generation by anaerobic digestion using semi-continuous feeding system. Obtained data show that populations of anaerobic saccharolytic, proteolytic, cellulose decomposers and acid producing bacteria were higher records at 20 days hydraulic retention time (HRT) than either 30 or 40 days HRTs. Highest concentration of volatile fatty acids (VFA) was obtained when the HRT and feeding rate were 20 days and 2 liter/day, respectively. The pH values which ranged form 7.17-7.72 were favourable to biogas generation and methane production and this was observed at different fermentation periods. The loss percentages in total and volatile solids were increased with the increase of hydraulic retention time. The highest rates of biogas production were observed during the first fermentation turn and this was true at various hydraulic retention times. Average methane percentage was higher at 20 days HRT compared to the methane percentages those observed at HRTs of 30 and 40 days. Highest rates of biogas and methane production per kg volatile solids either added or consumed were observed during the first fermentation turn. Biogas and methane production rates were higher at 20 days HRT during the first turn than other HRTs and fermentation turns used during the study.

Key words: Biogas, Artichoke, Anaerobic digestion, Semi-continuous feeding

Agro-industrial wastes like fruits and vegetables wastes are produced in large quantities amounted to 25-53% of raw materials (Hamza, 1989) and their disposal is a major problem confronting all agro-industrial establishment because of their high biodegradability. Organic matter wastes from certain types of crops are roundly produced all over the year, i.e. tomato, artichoke, legumes, jew's mallow, carrot and citrus. For example, the solid waste materials of El-Nasr company (one of the largest food industry establishment in Egypt) amounted to 7753 tons/year with a biodegradable fraction approaches to 65% of their total weight (El-Shimi et al 1992). A possible alternative for stabilization of these residues may be biodegraded by various anaerobic bacteria for biogas formation by methanogenic bacteria. Biogas generation and changes in total and volatile solids, volatile fatty acids and ammoniacal nitrogen were investigated for carrot, orange, phaseolus, pea and tomato wastes by (El-Shimi et al 1992). They found that biogas generation exhibited greater rates in the first fermentation cycle than the second fermentation one. Hydraulic retention time (HRT) and loading rate are the most important factors for biogas production stability. Hence, for semi continuous feeding system the HRT should be generally within a period of 3-4 weeks. HRT is in fact a design parameter and can be changed according to size of the digester, type of organic wastes and temperature of fermentation. Sarada and Joseph (1994) studied the biogas production during anaerobic digestion of tomato processing wastes and found that high yield of total biogas and methane were obtained at 24 days The present study aimed to investigate the effect of hydraulic retention time and HRT. loading rate on biogas production from Artichoke processing wastes by using semicontinuous feeding system.

# **Materials and Methods**

### Materials

Artichoke processing wastes were collected from the united company for food industry (Montana) in quantities enough to conduct the experiments. The wastes were well mixed and random samples were taken for initial analysis (Table, 1). Artichoke processing wastes were divided into portions, packed in plastic bags and frozen at-20°C until used.

Cattle dung was collected from the experiment station at Faculty of Agriculture, Moshtohor, Zagazig Univ., Banha Branch

| - U. C. AQL 11       | Unit   | Raw       | materials   |
|----------------------|--------|-----------|-------------|
| Parameters           |        | Artichoke | Cattle dung |
| pH                   | -      | 5.31      | 8.10        |
| Ammoniacal nitrogen  | ppm    | 61.01     | 560.13      |
| Volatile fatty acids | meq./l | 175.54    | 119.20      |
| Total solids         | %      | 12.68     | 16.03       |
| Volatile solids      | %      | 92.33     | 81.78       |
| Organic carbon       | %      | 53.55     | 47.43       |
| Total nitrogen       | %      | 2.09      | 1.70        |
| Total phosphorus     | %      | 0.50      | 1.30        |
| Total potassium      | %      | 1.55      | 0.94        |
| C/N ratio            |        | 25.62 :1  | 27.9:1      |

Table 1. Initial chemical analysis of raw materials used in the study,

Digesters: Anaerobic contact digester was used in this experiment. The digester had a total volume 60 liters with an active volume 40 liters.

### **Experimental procedures:**

Biogas production from Artichoke processing wastes.

Three units of anaerobic contact digester were used to study the biomethanation process of Artichokes processing wastes under three different hydraulic retention times (HRTs) being 20, 30 and 40 days. The digesters were firstly loaded with cattle dung of about 5% total solids (TS), which used as a seeding starter. Then, the digesters were incubated in a walk-in-incubator adjusted at  $35\pm1^{\circ}$ C till no more biogas was produced. Thereafter, a portion of the frozen Artichoke processing wastes was successively taken out, left to thaw at room temperature and mixed with tap water to appropriate solid concentration of 5%. The digesters were daily fed on a down-flow system according to the daily feeding rate per digester.

#### Determinations.

Volume of biogas produced was daily measured while its content of  $CH_4$  was determined every 2, 3 and 4 days according to HRTs. Total and volatile solids as well as macro and micro – elements were also determined at the beginning and end of digestion process. Chemical and bacteriological determinations were conducted as follows:

#### Chemical determinations.

Biogas production rate was estimated by measuring the height of the movable gas holder multiplied by surface area of digester to give the amount of the generated gas every day according to (Maramba *et al* 1978). Methane content was determined by gasliquid chromatography according to Wujick and Jewell method (1980). Carbon dioxide (CO<sub>2</sub>) content was estimated by means of Orsat's apparatus, using 33% potassium hydroxide solution for CO<sub>2</sub> absorption as the method described by Hamilton and Stephen (1964). Total solids, volatile solids, organic carbon, total phosphorus, microelements and volatile fatty acids (VFAs) were determined according to the standard method recommended by APHA (1992). Hydrogen ion concentration was directly measured by using 1: 5; slurry: water mixture; using glass electrode pH meter. Ammoniacal nitrogen and total nitrogen were determined according to Black *et al* (1965). Total potassium was determined according to Dewis and Freitas (1970).

Bacteriological analysis.

Anaerobic saccharolytic bacteria were counted on skim milk liquid medium, anaerobic proteolytic bacteria were counted on ox heart liquid medium and acid producing bacteria were counted on nutrient broth medium according to Cunningham (1954). Anaerobic cellulose decomposers were counted on Omelianskey's medium according to Allen (1959).

Counts of abovementioned groups of bacteria were estimated by using Most Probable Number (MPN) technique (Cochran, 1950).

# **Results and Discussion**

Biogas production from Artichoke processing wastes using semi-continuous feeding system:

Artichoke processing wastes were used to evaluate the biogas production by anaerobic digestion in contact digesters (60-L capacity). Three digesters were firstly loaded with the cattle dung as a starter. Then, the digesters were daily fed with a mixture of Artichoke processing wastes and water to reach 5% total solids. The loading rates were 2, 1.33 and 1 liter/day for 20, 30 and 40 days, respectively. This was done for three successive turns. The bacteriological changes, chemical changes, daily biogas production and methane percentage were determined throughout the anaerobic digestion course. Finally, biogas and methane production rates were calculated as either liter biogas or methane per kg volatile solids added or consumed.

1. Behaviour of various bacterial groups during anaerobic digestion of Artichoke processing wastes.

1.1. Behaviour of anaerobic saccharolytic bacteria.

The periodical changes of hetero and homo anaerobic saccharolytic bacteria during anaerobic digestion of Artichoke processing wastes are shown in Table (2). Obtained results show that when HRT was 20 days, counts of anaerobic saccharolytic bacteria (Hetero and Homo) were higher over the first turn than the either second or the third turn of fermentation course. On the other hand, counts of anaerobic heterosaccharolytic bacteria were higher during 1<sup>st</sup> turn than 2<sup>nd</sup> and 3<sup>rd</sup> turns when the HRT was 30 days whereas, counts of anaerobic homo-saccharolytic bacteria were slightly differed over the three turns throughout the anaerobic digestion of Artichoke processing wastes.

Similar performances of anaerobic saccharolytic bacteria (Hetero and Homosaccharolytic) at 40 days HRT were observed during various anaerobic digestion periods throughout the three turns of fermentation course.

The higher activities of anaerobic saccharolytic bacteria recorded when digesters were operated at HRT of 20 days compared to 30 and 40 days is most probably due to the higher feeding rate at HRT of 20 days (2.0 liter/day) than those used at 30 days (1.33 liter/day) and 40 days (1.0 liter/day).

1. 2. Behaviour of anaerobic proteolytic bacteria.

The periodical changes of anaerobic proteolytic bacteria are recorded in Table (2). Data show that at 20 days HRT, the highest counts of anaerobic proteolytic bacteria were observed during the first turn of fermentation  $(4.0-4.8 \times 10^4 \text{ cells / g dry weight})$ .

Counts of these bacteria decreased during the second and third turns of fermentation  $(1.2 \times 10^{-4} \text{ cells / g dry weight})$ . On the other hand, counts of anaerobic proteolytic were increased with increasing the feeding rate with the highest counts  $(4.8 \times 10^{-4} \text{ cells / g dry})$ 

| Digestion period<br>(days) | Anaerobic<br>Hetero | Saccharolytic<br>Homo | Anaerobic<br>proteolytic | Anaerobic<br>cellulose<br>decomposers | Anaerobic<br>acid<br>producers |
|----------------------------|---------------------|-----------------------|--------------------------|---------------------------------------|--------------------------------|
| A                          | HRT, 20             | days & feeding        | rate (2 liter/           | 'day)                                 |                                |
| 1 <sup>st</sup> turn       |                     |                       |                          |                                       |                                |
| Initial                    | 140                 | 54                    | 43                       | 16.28                                 | 730                            |
| 5 days                     | 150                 | 60                    | 46                       | 18.61                                 | 920                            |
| 10"                        | 180                 | 72                    | 44                       | 21.30                                 | 810                            |
| 20"                        | 170                 | 64                    | 48                       | 14.58                                 | 910                            |
| 2 <sup>nd</sup> turn       |                     |                       |                          |                                       |                                |
| 30"                        | 130                 | 42                    | 42                       | 14.10                                 | 320                            |
| 40"                        | 136                 | 48                    | 45                       | 16.81                                 | 430                            |
| 3 <sup>rd</sup> turn       |                     |                       |                          |                                       |                                |
| 50"                        | 140                 | 54                    | 41                       | 14.60                                 | 340                            |
| 60"                        | 126                 | 49                    | 44                       | 12.72                                 | 318                            |
|                            | HRT, 30 da          | ays & feeding r       | ate (1.33 liter          | r/day)                                |                                |
| 1 <sup>st</sup> turn       |                     |                       |                          |                                       |                                |
| Initial                    | 54                  | 13.30                 | 11.20                    | 10.56                                 | 230                            |
| 10 days                    | 52                  | 12.90                 | 11.40                    | 12.52                                 | 260                            |
| 20"                        | 57                  | 14.30                 | 11.90                    | 10.49                                 | 300                            |
| 30"                        | 55                  | 13.10                 | 11.30                    | 8.51                                  | 250                            |
| 2 <sup>nd</sup> turn       |                     |                       |                          |                                       |                                |
| 45"                        | 41                  | 14.2                  | 12.00                    | 8.46                                  | 110                            |
| 60"                        | 36                  | 13.7                  | 11.80                    | 9.43                                  | 160                            |
| 3 <sup>rd</sup> turn       |                     |                       |                          |                                       |                                |
| 75"                        | 33                  | 13.00                 | 12.10                    | 10.35                                 | 130                            |
| 90"                        | 38                  | 14.50                 | 12.50                    | 12.39                                 | 180                            |
|                            | HRT. 40             | days & feeding        | rate (1 liter/           | dav)                                  |                                |
| 1 <sup>st</sup> turn       |                     |                       |                          |                                       |                                |
| Initial                    | 17.90               | 11.10                 | 10.68                    | 9.31                                  | 143                            |
| 10 days                    | 16.20               | 10.74                 | 10.92                    | 8.37                                  | 176                            |
| 20"                        | 18.40               | 11.20                 | 11.10                    | 6.33                                  | 168                            |
| 40"                        | 17.00               | 10.80                 | 10.80                    | 9.35                                  | 184                            |
| 2 <sup>nd</sup> turn       |                     |                       |                          |                                       |                                |
| 45"                        | 19.20               | 11.50                 | 11.20                    | 6.23                                  | 110                            |
| 60"                        | 18.10               | 11.10                 | 10.70                    | 6.19                                  | 114                            |
| 75"                        | 16.00               | 10.91                 | 10.64                    | 8.16                                  | 154                            |
| 3 <sup>rd</sup> turn       |                     | 2.12.5                |                          |                                       |                                |
| 90"                        | 16.90               | 10.81                 | 10.69                    | 4.18                                  | 108                            |
| 105"                       | 19.20               | 11.30                 | 10.92                    | 6.24                                  | 112                            |
| 120"                       | 18.23               | 11.70                 | 11.40                    | 8.27                                  | 130                            |

Table 2. Periodical changes of microbial populations (x10<sup>3</sup>/g dry weight) during anaerobic digestion of Artichoke processing wastes under different HRT<sub>s</sub> for three successive turns.

weight) at feeding rate of 2 liter/day. Persistence of high counts of anaerobic proteolytic bacteria when the feeding rate was 2 liter/day compared to their counts at lower feeding rates i.e. 1.33 or 1 liter/day could be attributed to the continuous feeding of the digesters with enough amounts of fresh organic substances which provide ample

supply of organic N-compounds such as protein. Generally, the counts of anaerobic proteolytic bacteria ranged between  $10.64 \times 10^3 - 48 \times 10^3$  cells/g dry weight during anaerobic fermentation course. This result is in agreement with the findings of Siebert and Toerien (1969) and Hobson *et al* (1981) who reported a slightly higher counts of proteolytic bacteria ranged between  $10^4 - 10^5$  cells/ml during anaerobic digestion of organic wastes to biogas generation.

### 1. 3. Behaviour of anaerobic cellulose decomposers.

Data presented in Table (2) emphasize that counts of these bacteria gradually increased with time during anaerobic digestion period particularly over the first turn when the feeding rate was 2 liter/day and 20 days HRT. Counts of anaerobic cellulose decomposers were lower during the second and third turns compared with their counts during the first turn of fermentation course.

As regard to the counts of anaerobic cellulose decomposers at HRT 30 or 40 days, data recorded in Table (2) show that populations of these bacteria were lower than the counts recorded at 20 days HRT. It is obvious that counts of anaerobic cellulose decomposers were decreased with the decreasing of the daily feeding rate.

The obtained results show that counts of these bacteria ranged between  $4.18 \times 10^3$ -21.30x10<sup>3</sup> cell/g dry wieght. These results are in agreement with Hobson and Shaw (1974), El-Housseini (1983) and Zaghloul (1993) who found that populations of anaerobic cellulose decomposers ranged between  $2.1 \times 10^3 - 1.6 \times 10^4$  cells / g dry wieght during anaerobic digestion for biogas production from different organic residues.

## 1. 4. Behaviour of acid producing bacteria.

Data recorded in Table (2) show that the highest count of acid producers  $(9.1 \times 10^5$  cells/g dry weight) was observed at 20 days HRT and feeding rate 2 liter/day. While, the lowest count  $(1.0 \times 10^5$  cells/g dry weight) was observed at 40 days HRT and feeding rate 1 liter/day. It is obvious that counts of acid producers were higher during the first turn of fermentation than during either the second or third turns of fermentation. The same trend was observed during operation at three hydraulic retention times. The counts of these bacteria ranged between  $108 \times 10^3$  and  $920 \times 10^3$  cells/g dry weight. These results are in harmony with Scharer and Young (1979), Aly (1985) and Hanafy *et al* (1990) who found that counts of acid producing bacteria ranged between  $10^5$  and  $10^6$  cell/g dry mater when animal wastes and plant residues were anaerobically digested to biogas production. Moreover, the cellulolytic bacteria are generally present in fewer numbers  $10^3$  to  $10^5$  cell/ml than acidogenic population in the anaerobic digesters (Hobson and Shaw, 1974, Scharer and Young, 1979, El-Housseini, 1983 and Zaghloul, 1993).

It is important to mention that the populations of acid producing bacteria were parallel to the population of anaerobic cellulose decomposers that provide simple sugars for acid producing bacteria. The same trend was recorded at different hydraulic retention times as well as throughout different anaerobic digestion turns.

### 2. Chemical changes during anaerobic digestion of Artichoke processing wastes.

2.1. Changes in volatile fatty acids(VFA), ammoniacal nitrogen(NH4-N) and pH values.

Data presented in Table (3) show the highest concentrations of VFA during anaerobic digestion were obtained at 20 days HRT and feeding rate 2 liter / day. The same trend was observed at the three fermentation turns. Whereas, the lowest concentrations of VFA were obtained when the HRT and feeding rate were 40 days and 1 liter / day, respectively. Except for 40 days HRT, VFA concentrations were higher during the first turn of anaerobic digestion compared to the second and third fermentation turns. The increased production of volatile fatty acids at 20 days HRT is likely due to a possible activity of acid producing bacteria which showed an increase in their counts at hydraulic retention time of 20 days (Table, 2).

It is important to mention that the concentrations of VFA recorded in this study did not reach the levels reported to be toxic or even inhibitive to methane production by methanogenic bacteria as shown in (Table, 3). El-Shimi *et al* (1992) noticed the digesters containing carrot wastes showed the highest accumulation of VFA (187 meq. /l), whereas orange, legume and tomato wastes followed in order; producing concentrations of VFA 120.8, 58.1 and 48.3 meq. /l, respectively.

| Digestion period<br>(days) | VFA<br>(meg./l)  | NH <sub>4</sub> -N (ppm) | pH    |
|----------------------------|------------------|--------------------------|-------|
| HRT, 2                     | 0 days & feedin  | g rate (2 liter/day)     |       |
| 1 <sup>st</sup> turn       |                  | 8 ( )                    |       |
| Initial                    | 121.16           | 209.28                   | 7.17  |
| 5 days                     | 128.33           | 221.06                   | 7.29  |
| 10"                        | 136.07           | 237.84                   | 7.27  |
| 20" -                      | 141.20           | 244.08                   | 7.25  |
| 2 <sup>nd</sup> turn       |                  |                          |       |
| 30"                        | 114.03           | 240.19                   | 7.36  |
| 40"                        | 116.09           | 255.43                   | 7.38  |
| 3 <sup>rd</sup> turn       |                  |                          | 110.0 |
| 50"                        | 118.53           | 248.42                   | 7.42  |
| 60"                        | 116.94           | 259.76                   | 7.31  |
| HRT, 30                    | days & feeding   | rate (1.33 liter/day)    |       |
| 1 <sup>st</sup> turn       |                  |                          |       |
| Initial                    | 80.48            | 159.56                   | 7.23  |
| 10 days                    | 85.29            | 168.72                   | 7.28  |
| 20"                        | 88.47            | 172.30                   | 7.35  |
| 30"                        | 86.16            | 178.25                   | 7.29  |
| 2 <sup>nd</sup> turn       |                  |                          |       |
| 45"                        | 71.20            | 185.82                   | 7.38  |
| 60"                        | 76.08            | 180.23                   | 7.43  |
| 3 <sup>rd</sup> turn       |                  |                          |       |
| 75"                        | 70.68            | 181.34                   | 7.56  |
| 90"                        | 69.45            | 185.02                   | 7.44  |
| HRT, 40                    | 0 days & feeding | g rate (1 liter/day)     |       |
| 1 <sup>st</sup> turn       |                  |                          |       |
| Initial                    | 30.66            | 115.14                   | 7.30  |
| 10 days                    | 34.05            | 119.72                   | 7.38  |
| 20"                        | 37.12            | 134.16                   | 7.42  |
| 40"                        | 33.65            | 137.30                   | 7.61  |
| 2 <sup>nd</sup> turn       |                  |                          |       |
| 45"                        | 33.07            | 124.18                   | 7.25  |
| 60"                        | 35.98            | 135.24                   | 7.27  |
| 75"                        | 39.86            | 132.95                   | 7.41  |
| 3 <sup>rd</sup> turn       |                  |                          |       |
| 90"                        | 30.23            | 143.70                   | 7.72  |
| 105"                       | 37.26            | 141.38                   | 7.38  |
| 120"                       | 32.82            | 159.46                   | 7.66  |

 Table 3. Periodical changes of VFA, NH4-N and pH during anaerobic digestion of Artichoke processing wastes under different HRTs for three successive turns.

Data recorded in Table (3) show the concentration of ammoniacal nitrogen gradually increased with time over the fermentation period. This was true during each fermentation turn as well as at different HRT. The increase of  $NH_4$ -N concentration in digested slurry of Artichoke processing wastes with the progression in anaerobic digestion process could be attributed to the continuous feed of digesters with fresh organic materials. This could be explained by a continuous decomposition of organic

compounds and consequent continuous release of NH<sub>4</sub>-N at much higher rate than its rate of consumption. Moreover, concentrations of NH<sub>4</sub>-N were the highest when the HRT was 20 days and the daily feeding rate was 2 liter /day. On the contrary, the lowest concentrations of NH<sub>4</sub>-N were observed when the HRT was 40 days and the daily feeding rate was 1 liter /day. Accumulation of ammonia throughout the anaerobic digestion of Artichoke processing wastes at any hydraulic retention time as well as during any fermentation turn did not reach the level to be toxic or even inhibitive to methanogenic bacteria. Besides the role of NH<sub>4</sub><sup>+</sup> as a buffering agent in the fermentation medium since it neutralizes the acids produced throughout the acidogenic phase of the biodegradation process of the organic residues. It is also considered as the sole source of nitrogen for methanogenic bacteria. Chengdu (1979) reported that the lowest concentration of NH<sub>4</sub>-N during anaerobic fermentation of organic residues for biogas generation was determined by El-Shimi *et al* (1992) and Zaghloul (1993). They found that the NH<sub>4</sub>-N concentration increased in digested slurry with the progressive anaerobic digestion process.

Data presented in Table (3) also show the pH values slight increased with time over fermentation course. The same trend was observed at three hydraulic retention times under study. It is worthy to notice that the decreasing of pH values at the commencement of fermentation process is likely due to the increasing of volatile fatty acids concentration in the beginning of anaerobic digestion. In contrast, the increase of pH values during the late fermentation periods might be due to the higher concentrations of ammoniacal nitrogen.

Data recorded in Table (3) show the pH values were higher at 40 days HRT than either 20 days HRT or 30 days HRT. This result may be attributed to the lower recorded ratio between VFA and NH<sub>4</sub>-N when the HRT was 40 days. This result is in harmony with Paul and Beauchamp (1989) who reported that slurry pH was largely affected by VFA and total ammonia concentrations and increased as (VFAs) / (NH<sub>4</sub><sup>+</sup> +NH<sub>3</sub>) ratio decreased. Moreover, obtained data in this study showed a pH range which is favourable to biogas and methane production. The favourable pH values for methanogenic bacteria recorded by earlier investigators ranged between 6.5-8.05 (Sarada and Nand, 1989; El-Shimi *et al.* 1992 and Ghaly, 1996).

It is obvious that the hydrogen ion concentration recorded in the present study remained at the favourable level for methanogenic bacteria all over the fermentation course when the semi-continuous feeding system was used. This may be due to the continuous feeding of the digesters by fresh materials which was biodegraded by lytic microorganisms and the ammonium ion released neutralizes the acids produced by acid producing bacteria within the digesters. Therefore, it could be concluded that the fermentation process by using the semi-continuous feeding is more stable than the batch feeding method.

### 2.2. Changes in macro and micro-elements

The percentages of macro and micro-nutrients in digested slurry of Artichoke processing wastes were higher at the end of fermentation period compared to those recorded at the beginning of fermentation (Table, 4).

This increase is likely due to the consumption of total and volatile solids during anaerobic digestion by fermentative microorganisms to produce  $(CH_4 \text{ and } CO_2)$  and other gases.

Similar results were observed by Hanafy *et al* (1990), El-Shimi *et al* (1992) and Estefanous *et al* (1997) who reported that percentages of (N, P and K) were increased at the end of anaerobic digestion compared to the initial period. This was explained by a possible loss in total and volatile solids during conversion to  $CH_4$ , carbon dioxide and other gases.

# 2. 3. Changes in total and volatile solids.

The concentration of total and volatile solids decreased throughout fermentation course. Loss in these components was certainly due to conversion of organic substances into gases (CH<sub>4</sub> and CO<sub>2</sub>). The loss percentages in total and volatile solids increased with the increase in hydraulic retention time. This result was observed at three anaerobic digestion turns. On the other hand, data recorded in Table (5) emphasize that the loss percentages of total and volatile solids were higher during the second and third fermentation turns than the losses observed during the first fermentation turn. The conversion rates decreased during the second and third fermentation turns. Such results reflect instability in the fermentation process.

Table 4. Concentration of macro and micro-nutrients during anaerobic digestion of Artichoke processing wastes under different HRT<sub>s</sub> for three successive turns.

|                  | HRT, 20 days & feeding rate (2 liter/day) |       |                      |           |                      |       |  |  |
|------------------|---|-------|----------------------|-----------|----------------------|-------|--|--|
| Nutrients        | 1 <sup>st</sup> turn                      |       | 2 <sup>nd</sup> turn |           | 3 <sup>rd</sup> turn |       |  |  |
|                  | Initial                                   | Final | Initial              | Final     | Initial              | Final |  |  |
|                  |   |       | Macro-               | nutrients | 12.77                |       |  |  |
| Total nitorgen   | 2.42                                      | 2.74  | 2.51                 | 2.59      | 2.41                 | 2.52  |  |  |
| Total phosphorus | 1.20                                      | 1.24  | 1.19                 | 1.23      | 1.12                 | 1.24  |  |  |
| Total potassium  | 1.13                                      | 1.17  | 0.99                 | 1.11      | 1.09                 | 1.16  |  |  |
|                  |   |       | Micro-n              | utrients  |                      |       |  |  |
| Iron (ppm)       | 760                                       | 780   | 750                  | 785       | 783                  | 740   |  |  |
| Zinc (ppm)       | 64  | 69    | 66                   | 71        | 60                   | 67    |  |  |
| Manganese (ppm)  | 27  | 30    | 24                   | 29        | 28                   | 34    |  |  |
| Copper (ppm)     | 52  | 58    | 50                   | 54        | 56                   | 61    |  |  |

|                         | HRT, 30 days & feeding rate (1.33liter/day) |                 |                      |           |                      |       |  |  |  |  |
|-------------------------|---|-----------------|----------------------|-----------|----------------------|-------|--|--|--|--|
|                         | 1 <sup>st</sup> tu                          | rn              | 2 <sup>nd</sup> turn |           | 3 <sup>rd</sup> turn |       |  |  |  |  |
|                         | Initial                                     | Final           | Initial              | Final     | Initial              | Final |  |  |  |  |
| 1. 1                    |   | Macro-nutrients |                      |           |                      |       |  |  |  |  |
| Total nitorgen          | 2.35  | 2.29            | 2.33                 | 2.39      | 2.28                 | 2.37  |  |  |  |  |
| <b>Total phosphorus</b> | 0.80  | 0.87            | 0.76                 | 0.89      | 0.65                 | 0.93  |  |  |  |  |
| Total potassium         | 0.95  | 0.98            | 0.92                 | 0.96      | 0.86                 | 0.99  |  |  |  |  |
|                         |   |                 | Micro-               | nutrients |                      |       |  |  |  |  |
| Iron (ppm)              | 755   | 710             | 721                  | 713       | 724                  | 728   |  |  |  |  |
| Zinc (ppm)              | 52  | 58              | 51                   | 54        | 50                   | 59    |  |  |  |  |
| Manganese (ppm)         | 18  | 26              | 19                   | 24        | 22                   | 28    |  |  |  |  |
| Copper (ppm)            | 33  | 37              | 31                   | 35        | 34                   | 39    |  |  |  |  |

|  | HRT, 40 days & feeding rate (1 liter/day) |       |                      |           |                      |       |  |  |  |
|--|---|-------|----------------------|-----------|----------------------|-------|--|--|--|
|  | 1 <sup>st</sup> tu                        | rn    | 2 <sup>nd</sup> turn |           | 3 <sup>rd</sup> turn |       |  |  |  |
|  | Initial                                   | Final | Initial              | Final     | Initial              | Final |  |  |  |
| 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1 |   | 1.000 | Macr                 | o-nutrien | ts                   |       |  |  |  |
| Total nitorgen                           | 2.20                                      | 2.26  | 2.25                 | 2.29      | 2.17                 | 2.24  |  |  |  |
| <b>Total phosphorus</b>                  | 0.56                                      | 0.59  | 0.60                 | 0.68      | 0.54                 | 0.59  |  |  |  |
| Total potassium                          | 0.81                                      | 0.86  | 0.74                 | 0.79      | 0.80                 | 0.87  |  |  |  |
|  |   |       | Micr                 | o-nutrien | ts                   |       |  |  |  |
| Iron (ppm)                               | 600                                       | 610   | 619                  | 645       | 618                  | 640   |  |  |  |
| Zinc (ppm)                               | 31  | 42    | 33                   | 39        | 40                   | 46    |  |  |  |
| Manganese (ppm)                          | 16  | 19    | 17                   | 19        | 16                   | 20    |  |  |  |
| Copper (ppm)                             | 23  | 27    | 20                   | 24        | 26                   | 30    |  |  |  |

This was indicated by the lower rate of biogas generation and relatively larger accumulation of metabolites such as ammoniacal nitrogen. In addition, the decline in the extent of bioconversion could be due to changes in the bacterial growth rate resulting from loss of fermented solids from the digesters whenever the fresh slurry was added. Similar results were observed by Sax *et al* (1980), El-Shimi *et al* (1992), Zaghloul (1993) who reported that the loss rates of total and volatile solids were found to dependant on the application system, type of organic wastes, amendment with starter, fermentation period and temperature of the digester.

|        |                              |       |        | Total se        | olids (K             | g/digester) |         |                            |        |  |
|--------|------------------------------|-------|--------|-----------------|----------------------|-------------|---------|----------------------------|--------|--|
| HRT    | T <u>1<sup>st</sup> turn</u> |       |        |                 | 2 <sup>nd</sup> turn |             |         | <u>3<sup>rd</sup> turn</u> |        |  |
| (days) | Initial                      | Final | Loss % | Initial         | Final                | Loss %      | Initial | Final                      | Loss % |  |
| 20     | 1.992                        | 1.501 | 24.65  | 2.024           | 1.373                | 32.16       | 1.968   | 1.460                      | 25.81  |  |
| 30     | 2.000                        | 1.301 | 34.95  | 1.980           | 1.240                | 37.37       | 2.032   | 1.219                      | 40.01  |  |
| 40     | 1.980                        | 1.255 | 36.62  | 2.000           | 1.140                | 43.00       | 1.960   | 1.046                      | 46.63  |  |
|        |                              |       | Vol    | atile solid     | ls(Kg/dig            | gester)     |         |                            |        |  |
|        | 1 <sup>st</sup> turn         |       |        | 2 <sup>nd</sup> | turn                 |             |         | 3rd turn                   |        |  |
|        | Initial                      | Final | Loss % | Initial         | Final                | Loss %      | Initial | Final                      | Loss % |  |
| 20     | 1.839                        | 1.348 | 26.99  | 1.869           | 1.218                | 34.83       | 1.817   | 1.309                      | 27.96  |  |
| 30     | 1.847                        | 1.148 | 37.85  | 1.828           | 1.088                | 40.48       | 1.876   | 1.063                      | 43.34  |  |
| 40     | 1.828                        | 1.103 | 39.66  | 1.847           | 0.987                | 46.56       | 1.810   | 0.896                      | 50.50  |  |

Table 5. Changes in total and volatile solids during anaerobic digestion of Artichoke processing wastes under different HRT<sub>s</sub> for three successive turns.

2.4. Evaluation of biogas produced and its methane content.

Data graphically illustrated in Figure (1) show that the fermented materials generated biogas after the first day of fermentation. The early generation of biogas that occurred in this study is likely due to enriching the digesters with the fermented slurry serving as a starter at the onset. Such an inoculum of animal origin provided the fresh feedstock with active facultative and strict anaerobic bacteria together with their energy and nutritional requirements. In this concern, Hobson *et al* (1981) and Marty (1984) found that the ruminant wastes contain active anaerobic acid producers and methane producing bacteria. Also, Stevenson (1982) reported that utilization of the spent slurry of digested cattle dung as a seeding starter with plant residues subjected to fermentation had been found to hasten the biomethanation process and thus shorten the initial period required for the flammable gas to be produced.

The cumulative biogas yield from the fermented wastes decreased with the increase in HRT (Figure, 1). This was true during various fermentation turns. For example, total biogas yields during the first turn were as follows; 280, 272 and 186 liter / digester when the HRTs were 20, 30 and 40 days, respectively. Average rates of volumetric biogas evolution (liter / liter fermented material) were 0.35, 0.23 and 0.12 for the daily feeding rates of 2, 1.33 and 1 liter / day, respectively. This was expected since the fermentative bacteria find enough amounts from their nutritional requirements at high feeding rates and this was consequently reflected on their metabolites.

Regarding the daily biogas production rate from the anaerobic digestion of Artichoke processing wastes, obtained data showed a fluctuation during fermentation course. The same trend was observed at different investigated hydraulic retention times. The higher cumulative biogas yield which observed at 20 and 30 days HRT compared to the cumulative biogas obtained at 40 days HRT could be attributed to the high activity of microbial populations i.e. anaerobic saccharolytic, anaerobic proteolytic, anaerobic cellulose decomposers and anaerobic acid producers which had recorded higher populations at 20 and 30 days HRT than their counts at 40 days HRT (Table, 2).

With respect to total biogas yield during different fermentation turns, obtained data show that the total biogas generation rates during the second and third turns were lower than the first fermentation turn. This was true at different hydraulic retention times. The highest rates of biogas produced during the first turn of digestion period could be mainly attributed to enriching the digesters with previously digested cattle dung serving as a starter at the fermentation commencement. Whereas, the lower rates of biogas yield which appeared during the second and third turns might be due to an outflux of a large proportion of the bacterial agents and their nutrients from the digesters via the daily removal of the effluent and / or due to a relatively high accumulation of  $NH_4$ -N in the digesters which appeared directly proportional to time. Similar results were observed by El-Shimi *et al* (1992), Zagloul (1993) and Estefanous *et al* (1997) who mentioned that the biogas generation from organic wastes using the semi-continuous feeding system exhibited greater rates in the first turn compared with the second turn of fermentation course under the same conditions.

The average of daily biogas produced from the anaerobic digestion of Artichoke processing wastes at different HRTs as well as throughout the different fermentation turns ranged between 0.12-0.35 liter biogas / liter fermented material / day. In this concern, Cooney and Wise (1975) reported that thermophilic fermentation produced biogas at a rate of 0.31 volume gas per volume digested material per day (V / V / day) being greater than gas production of mesophilic operated digesters which was only 0.21 V / V / day. Stout and Loudon (1977) showed that increasing the loading rate from 1.17 to 5.29 Kg dry solids m<sup>-3</sup> of digester volume day<sup>-1</sup> increased the volume of biogas from 0.26 to 0.47 biogas m<sup>-3</sup> digesting slurry day<sup>-1</sup> (V / V / day). Also, biomethanation of banana peel and pineapple wastes was studied by Barida *et al* (1996) at various HRTs and showed that a higher rate of biogas production was observed at lower retention time.





2.5. Methane yield and methane percentage.

Data graphically illustrated in Figure (2) show that the daily methane production rate was higher at 20 days HRT than that observed at 30 or 40 days HRTs. This trend was observed throughout the different fermentation turns. Moreover, average of daily methane yield was higher during the first turn than that obtained for the second or third fermentation turns and that was true at all different hydraulic retention times.



Figure (2): Periodical changes of methane production and methane percentages during anaerobic digestion of Artichoke processing wastes under different HRTs for three successive turns.

The average of methane percentage was higher at 20 days HRT compared to that observed at 30 and 40 days HRTs during the first and second fermentation turns. While, the average methane percentage during the third fermentation turn was higher at 30 days HRT compared with that recorded at other hydraulic retention times. The higher methane yield observed at 20 days HRT during the first fermentation turn is likely due to the high activity of lytic and fermentative bacteria (Table, 2). Under such conditions much higher concentrations of volatile fatty acids which represent the ample supply for methanogenic bacteria were observed at 20 days HRT - during the first fermentation turn. Similar results were observed by El-Shimi *et al* (1992) and Zaghloul (1993) who reported that the methane yield and methane percentage decreased during the second and third turns when both food processing wastes and agricultural residues were anaerobically digested for biogas production.

Also, biomethanation of banana peel and pineapple wastes was studied by Barida *et al* (1996) at various HRTs and showed that a higher rate of biogas production was observed at lower retention time.

2.6. Rate of organic substrate conversion to biogas and methane.

Data presented in Table (6) clearly show that the highest rates of biogas and methane production per kg volatile solids either added or consumed were observed during the first fermentation turn and much lower rates were observed during the third fermentation turn. Moreover, it is obvious that the biogas and methane production rates were higher at 20 days HRT than other hydraulic retention times.

The higher production rates of biogas and methane observed during the first turn of anaerobic digestion of Artichoke processing as well as at 20 days HRT could be attributed to the high activity of lytic and fermentative bacteria viz., anaerobic saccharolytic, proteolytic, cellulose decomposers and acid producing bacteria recorded under such conditions (Table, 2).

In this concern, Sarada and Joseph (1994) found that the highest yield of biogas mixture and methane were obtained at 24 days HRT during anaerobic digestion of tomato processing waste. Linke (1997) reported that the optimum hydraulic retention time for biogas generation from the anaerobic digestion of pig slurry and fat wastes was 20 days.

| HRT    | 1              | Biogas and methane production (L/kg VS added) |            |               |                |         |  |  |  |  |
|--------|----------------|---|------------|---------------|----------------|---------|--|--|--|--|
| (days) | The first turn |   | The seco   | ond turn      | The third turn |         |  |  |  |  |
|        | Biogas         | Methane                                       | Biogas     | Methane       | Biogas         | Methane |  |  |  |  |
| 20     | 152.04         | 101.62  | 140.24     | 92.00         | 133.63         | 85.52   |  |  |  |  |
| 30     | 147.21         | 95.83   | 142.45     | 92.45         | 126.76         | 81.63   |  |  |  |  |
| 40     | 101.59         | 65.63   | 95.72      | 60.69         | 92.21          | 58.28   |  |  |  |  |
|        | Bi             | ogas and me                                   | thane prod | luction (L/kg | VS consu       | med)    |  |  |  |  |
| 20     | 569.45         | 379.25  | 477.95     | 305.89        | 402.61         | 264.11  |  |  |  |  |
| 30     | 388.98         | 253.23  | 351.89     | 228.38        | 292.50         | 188.37  |  |  |  |  |
| 40     | 256.14         | 165.47  | 205.58     | 130.34        | 182.60         | 115.40  |  |  |  |  |

Table 6. Biogas and methane production rates during anaerobic digestion of Artichoke processing wastes under different HRT<sub>s</sub> for three successive turns.

Respecting the relation between biogas and methane production and fermentation turns, El-Shimi *et al* (1992) and Zaghloul (1993) found that the biogas and methane production rates were relatively lower during the second and third fermentation turns compared to the first fermentation turn.

# References

Allen, O. N. (1959). Experiments in Soil Bacteriology. 3rd Ed. Burges publ. Co. U.S.A.

- Aly, B. E. (1985).Bioenergy from organic residues for rural Egypt. Ph.D. Thesis, Fac. of Agriculture, Ain Shams Univ.
- American Public Health Association (APHA) (1992). Standard methods for the examination of water and wastewater. 18<sup>th</sup>. Ed. Washington, D. C.
- Barida, N.; Somayaji, D. and Khanna. S. (1996). Biomethanation of banana peel and pineapple wastes. Biores. Technol., 58: 73-76.
- Black, C. A.; Evans, D. O.; Ensminger, L. E.; White, J. L.; Clark, F. E. and Dinaure, R.C. (1965). Methods of Soil Analysis. II-Chemical and Microbiological Properties. Am. Soc. Agr. Inc. Madison, Wisconsin, U.S.A.
- Chengdu, S. (1979).Biogas technology and utilization. Sichuan provinical Office of Biogas Development, China.
- Cochran, W. G. (1950). Estimation of bacterial densities by means of the most probable number. Biometrics, (6): 102-116.
- Cooney, C. L. and Wise, D. L. (1975). Thermophilic anaerobic digestion of solid wastes for fuel gas production. Biotech., Bioenging, (17): 1119-1135.
- Cunningham, A. (1954). Practical Bacteriology. 2<sup>nd</sup> Ed. Oliver and Boyed, Edinburgh and London.
- Dewis, G. and Freitas, F. (1970). Physical and chemical methods of soil and water analysis. F.A.O., Bull., No. (10).
- El-Housseini, M. (1983). Fermentation of city refuses under anaerobic conditions. Ph.D. Thesis, Ain Shams Univ. Cairo, Egypt.
- El-Shimi, S. A.; El-Housseini, M.; Ali, B. E. and El-Shinnawi, M. M. (1992). Biogas generation from food-processing wastes. Resources conservation and Recycling (6): 315-327.
- Estefanous, A. N.; Fahmy, Soheir; Mikhaeel, F. T. and El-Shimi, S. A. (1997). Effect of aerobic pretreatment on anaerobic digestion of maize stalks. Proceedings of the 9<sup>th</sup> Conference of Microbiology, Cairo, March, 25-27.
- Ghaly, A. E. (1996). A comparative study of anaerobic digestion of acid cheese whey and dairy manure in a two stage reactor. Biores. Technol., 58: 61-72.
- Hamilton, L. F. and Stephen, G. S. (1964). Quantitative chemical analysis. The Macmillan company, New York, pp. 454-459.
- Hamza, A. (1989). Utilization of agro-industerial residues in Alexandria: Experience and prospects. Biol. Wastes, 29, 107-121.
- Hanafy, Ehsan, A.; Newigy, N. A.; El-Housseini, M. and Estefanous, A. N. (1990). Chemical and microbiological changes during composting of city refuse. Agricultural research review, 68 (2): 347-357.
- Hobson, P. N., and Shaw, B. G. (1974). The anaerobic digestion of waste from an intensive pig unit. Water Res., 8, 507-516.
- Hobson, P. N.; Bousfield, S. and Summers, R. (1981). Methane production from agricultural and domestic wastes. App. Sci. Pub. Ltd., London.
- Linke, B. (1997). Disposal of organic wastes through co-digestion. Landtechnik, 52 (2): 82-83.
- Maramba, F. D.; Obias, E. D.; Julian, B.; Taganas, C.; Alumbro, R. D.; and Judan, A. A. (1978). Biogas and waste recycling, the Philippine experience. Maya farms division, liberty flour mills, Inc. Metro Manila, Philippines.
- Marty, B. (1984). Microbiology of anaerobic digestion. Anaerobic digestion of sewage sludge and organic agricultural wastes. Proceeding of a Seminar Organized by the Commission of the European Communities, Research and Development, Environment Research Programme, Athens, Greece, 14-15 may, 72-90.
- Paul, J. W., and Beauchamp, E. G. (1989). Relationship between volatile fatty acids, total ammonia and pH in manure slurries. Biol. Wastes, 29, 313-318.
- Sarada, R. and Nand, K. (1989). Start-up of anaerobic digestion of tomato-processing wastes for methane generation. Biol. Wastes. 30: 231-237.

- Sarada, R. and Joseph, R. (1994). Studies on factors influencing methane production from tomato-processing waste. Biores. Technol., 47, 55-57.
- Sax, R. I.; Morice, H. and Pett, K. C. (1980). Production of biogas from wastewater of food processing industries. In Conf. Industrial Energy Conservation Technology and Exhibition, Houston.
- Scharer, M. F. and Young, J. C. (1979). Effect of media design on the performance of fixed bed anaerobic reactors. Proceedings of the international association on water pollution research seminar on anaerobic treatment of wastewater in fixed film reactors., 16-18 June. Copenhagen.
- Siebert, M. L. and Toerien, D. F. (1969). The proteolytic bacteria present in the anaerobic digestion of raw sewage sludge in the anaerobic digestion of raw sewage sludge. Water Res., 3, 241.
- Stevenson, F. J. (1982). Humus chemistry, Genesis, Composition and Reactions. John Willy & Sons. New York.
- Stout, B. A. and Loudon, T. L. (1977). Energy from organic residues. FAO / UNDP Seminar on Residue Utilization, Management of Agricultural and Agro-industrial Residues, Rome, Italy.
- Wujick, W. J. and Jewell, W. J. (1980). Dry anaerobic fermentation. Biotechnology and bioengineering Symp. No. 10, p. 43, Jon Willey & Sons, New York.
- Zaghloul, R. A. (1993). Bioconversion of agricultural residues to biogas products. Ph.D. Thesis, Fac. Agric. Moshtohor, Zagazig University, Egypt.

هذه الدراسة .

فقد أظهرت النتائج أن أعلى معدل لإنتاج البيوجاز والميثان لوحظ عند استخدام مدة تخمير عشرون يوماً وذلك عند بخصوص معدلات إنتاج البيوجاز والميثان على أساس كمية المادة العضوية المضافة أو المستهلة بالكيلوجرام دورة التخمير الأولى لمخلفات التصنيم الغذائي للخرشوف مقارنة بفترات ودورات التخمير الأخرى التي استخدمت في

تخمير ثلاثون أو أربعون يوما .

أعلي نسبة للميثان فى البيوجاز الناتج لوحظت عند استخدام مدة تخمير عشرون يوماً وذلك بالمقارنة بمدة

كذلك أوضحت النتائج أن درجات الـــــ PHفى محلول التخمير والتي تراوحت بين ٧,٧٢-٧,٧٢ خلال فترات التخمير المختلفة لمخلفات التصنيح الغذائي للخرشوف لم تصل إلى مستوي السمية أو التثبيط حيث كانت ملائمة لإنتاج البيوجاز بواسطة بكتريا الميثان .

للسليولوز وكذلك البكتريا المنتجة للأحماض ظهرت بأعداد عالية عندما كانت مدة التخمير عشرون يومأ مقارنة بمدة وقد أوضحت النتائج أن أعداد البكتريا اللاهوائية المحللة للكربوهيدرات وتلك المحللة للبروتينات والمحللة تخمير ثلاثون أو أربعون يوما . أعلى تركيز للأحماض العضوية المتطايرة في محلول التخمير ظهر عند استخدام مدة تخمير عشرون يوما ومعدل تغذية ٢ لتر/ يوم.

أثناء الهضم اللاهوائي لمخلفات التصنيع الغذائي للخرشوف باستخدام نظام التغذية شبه المستمرة وذلك فى مخمرات في هذا البحث درس معدلات إنتاج البيوجاز ومحتواه من الميثان وكذلك التغيرات البكتريولوجية والكيماوية سعة ٦٠ لتر خلال ثلاث فترات تخمير مختلفة هي ٢٠، ٣٠، ٤٠ يوما لثلاث دورات متعاقبة .

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إنتاج البيوجاز من مخلفات التصنيع الغذائي للخرشوف باستخدام نظام التغذية شبه المستمرة .

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